REMARKS

Applicants would like to thank Examiner Yong Pak and her supervisor Ponnathapu Achutamurthy for a productive discussion regarding the amendments and arguments set forth herein, during the in-person interview on June 29, 2004, attended by Examiners Pak and Achutamurthy, the undersigned, and the undersigned's associate Dr. Anna Solowiej. As acknowledged by both Examiner Pak and Examiner Achutamurthy, the claims appear to be allowable over the prior art of record.

Claims 1-7, 20-29 and 34-82 are pending in the case, claims 30-33 having been cancelled by the above amendment and new claims 47-82 added. Claims 20-29 are withdrawn pursuant to a restriction requirement, but are subject to rejoinder once the composition claims currently under examination are deemed allowable. Thus, Applicants have amended certain of the withdrawn method claims (claims 20 to 25) and have added new method claims 47-82 (modeled on original claims 20-28) to ensure the claims to be rejoined are in condition for allowance. The amendments to claims 1, 4, 34, 35, 41, and 43 merely clarify the intended scope of the claims as limited to mutants with changes at defined positions of a particular enzyme represented by SEQ ID NO:2. These amendments neither narrow the claims nor add new matter.

Applicants note that in the present Office action, all of the grounds for rejection set forth in the previous Office action dated August 26, 2003, were withdrawn, save one: the rejection of the claims under 35 USC §103(a) as unpatentably obvious over Galkin et al. in view of Slusarczyk et al. and Tishkov et al. This rejection is reasserted in the present Office action against claims 1-7 and 30-46. As claims 30-33 are newly cancelled, it is moot with respect to them. Applicants traverse the rejection as applied to the remaining claims 1-7 and 34-46, addressing them in two (overlapping) sets: first claims 1-7 and 41-46, and then claims 34-46.

Claims 1-7 and 41-46

As the Examiner is no doubt aware, a *prima facie* case of obviousness must establish two things: first, that the prior art provides the motivation to make the claimed invention exactly as claimed (*i.e.*, including all limitations); and second, that the prior art demonstrates that one of ordinary skill would have had an expectation that the claimed invention will be successful. It is

not enough to argue that the prior art taught that experimentation in a general field (e.g., modifying cysteine residues of formate dehydrogenases) would be a potentially useful thing to do. The prior art must also provide the suggestion that the invention *as claimed* should be made, and further, an expectation it would be likely to work.

Each of claims 1-7 and 41-46 requires that the claimed polypeptide have a mutation at the position corresponding to Cys-146 of SEQ ID NO:2. All of the cited references discuss mutagenizing various residues of various formate dehydrogenases of various organisms, but not a single one of the cited references suggests mutating a Cys residue that could be said to correspond to Cys-146 of SEQ ID NO:2. Indeed, the Examiner does not attempt to argue that the prior art teaches mutagenizing Cys-146 *per se* of SEQ ID NO:2 *per se*, nor even the corresponding cysteine of any other formate dehydrogenase. Instead, the Office action appears to take the position that a motivation corresponding precisely to the claim limitations is unnecessary, so long as the art teaches the general concept that it may be useful to replace cysteine residues *in general*, in formate dehydrogenases *in general*, as well as a way to do so. ("Since there is suggestion and expectation that modification of a cysteine residue will increase the enzyme's activity, there is sufficient motivation of making modifying [*sic*] the cysteine residues of SEQ ID NO:2. It is not uncommon nor an undue burden to modify seven residues in an enzyme and site-directed mutagenesis is very routine in the art, as well as generating mutants having double/triple mutations." Office action, page 4.) No rationale is offered for selecting Cys-146 in particular, of SEQ ID NO:2 in particular. Thus, what the Office action styles as the requisite "motivation" is plainly not in accordance with established U.S. law. According to the Federal Circuit, "A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out." *In re Deuel*, 51 F.3d 1552, 1559, 34 USPQ2d 1210, 1216 (Fed. Cir. 1995). And here the claims are certainly drawn to a very particular result. A proper obviousness rejection must indicate where the prior art would motivate the skilled person to make the invention as claimed, *i.e.*, including all limitations of the claim, and not merely sketch out a research plan that is in the same general field and might or might not end up with the claimed invention.

Furthermore, in order to maintain a rejection for obviousness, the Examiner must indicate where the expectation of success is disclosed in the prior art. The first reference, Galkin et al., did not even attempt to mutagenize any cysteine residues, so does nothing to contribute to an expectation of success in mutagenizing Cys-146. Tishkov et al. modified Cys-255 of a *Pseudomonas sp.* formate dehydrogenase, but did not address, even indirectly, whether modifying the counterpart to Cys-146 of SEQ ID NO:2 would have been of any use. There is certainly no basis for assuming that a beneficial result in mutagenizing at one Cys residue would be reasonably predictive of success in mutagenizing another Cys residue over 100 residues away.

The Examiner relies primarily on Slusarczyk et al. to supply the expectation of success. According to the Office Action, Slusarczyk et al. teaches mutagenizing "all the cysteine residues" in the primary structure of a formate dehydrogenase from *Candida boidinii*, "to increase the enzyme's stability by alleviating any thiol-coupled inactivation." Slusarczyk et al. does indeed disclose the mutagenization of "all" of the (two!) Cys residues of the *Candida boidinii* enzyme (Cys-23 and Cys-262), but goes on to say that mutagenizing one of them, Cys-262, did not increase the stability of the enzyme at all. In fact, under some test conditions, replacing Cys-262 alone actually decreased the enzyme's activity relative to wildtype (see, e.g., Figs. 9 and 11), and replacing both Cys-23 and Cys-262 decreased the activity relative to replacing Cys-23 alone (see, e.g., Figs. 10 and 11). Thus, it is a mischaracterization of the teachings of Slusarczyk et al. to say that these authors "mutagenized all the cysteine residues in the primary structure to increase the enzyme's stability by alleviating any thiol-coupled inactivation." A more accurate reading of Slusarczyk et al. instructs the reader that (1) mutagenizing Cys-23 might be a good thing to try, but (2) mutagenizing Cys-262 will not be successful, and so (3) one can't extrapolate an improvement in activity upon mutagenizing one cysteine residue in a given protein to an expectation of improvement in activity upon mutagenizing another. Since SEQ ID NO:2 of the present invention does not even possess a Cys residue that aligns with Slusarczyk et al's Cys-23, and there is no counterpart to the Cys-146 residue of SEQ ID NO:2 in Slusarczyk et al.'s enzyme, the sole take-home message of this reference relevant to the presently claimed enzyme is the general unpredictability of success in

mutagenizing Cys residues in formate dehydrogenases. This makes it clear that there could have been no expectation that mutagenizing Cys-146 would be beneficial.

The Office action acknowledges that modifying some cysteine residues of a formate dehydrogenase might lead to a decrease in activity:

> [One] of ordinary skill in the art would recognize that modification of Cys-262 [of Slusarczyk et al.'s enzyme] might lead to disruption of the protein structure thereby decreasing its activity. Therefore, it is not unexpected that some mutants will have a decreased activity compared to the wildtype enzyme. Office action, page 4.

This admission that some mutants will likely have decreased activity (as did one of Slysarczyk et al.'s mutants, at Cys-262), coupled with a total lack of prediction in the art of what would happen if Cys-146 of a formate dehydrogenase is mutated, establishes conclusively that there was no expectation of success in the art regarding modifying Cys-146, and furthermore that the Examiner realizes it. It appears that the Examiner believes that all that is required to support an obviousness rejection of the present narrowly-drawn claims is an expectation that SOME mutants MIGHT have activity. Even if it were, as the Examiner seems to believe, obvious to try experimenting with modifying <u>all</u> of the cysteine residues of SEQ ID NO:2, even the Examiner will agree it was not predictable that modifying <u>Cys-146 in particular</u> would yield an enzyme that was active. Even less was it predictable that modifying this particular residue would yield a mutant that not only is active, but in fact also possesses beneficial properties never before recognized in any formate dehydrogenase mutant. Thus, the rejection of claims 1-7 and 41-46 as obvious fails to meet the two basic requirements for any *prima facie* obviousness rejection: first, it does not establish a motive in the prior art to modify Cys-146, in particular, of SEQ ID NO:2, in particular; and second, it does not establish an expectation of success that the claimed mutant will be active.

Claims 34-46

Claims 34-46 all specify that the cysteine residues at both positions 6 and 256 of SEQ ID NO:2 be altered.[1] While Tishkov et al. (working with a *Pseudomonas* formate dehydrogenase closely related to SEQ ID NO:2) did try making substitutions at Cys-255, a position that corresponds to Cys-256 of SEQ ID NO:2, they did not make a second substitution anywhere in their enzyme. Nor did they suggest any reason to do so. They did state that Cys-5 (corresponding to Cys-6 of SEQ ID NO:2) is likely to be "essential for enzyme activity," which, as previously pointed out by Applicants, provides a reason not to alter it if preserving activity is the goal. Tishkov et al. also stated that "Data obtained show that Cys255 is unique residue for providing both enzyme thermostability and catalytically optimal binding of coenzyme." (abstract; emphasis added) This focus on the importance of Cys255 is based in part on their comparison of the *Pseudomonas* enzyme's sequence to that of a yeast formate dehydrogenase with 48.9% homology. As noted on page 981 of the reference, only two of the seven *Pseudomonas* enzyme's cysteines, *i.e.*, Cys-255 and Cys-288, line up with cysteines of the yeast enzyme; and of the two, only Cys-255 is in a region that participates in binding of coenzyme or substrate. Cys-5 of the *Pseudomonas* enzyme does not even have an equivalent in the yeast enzyme, and does not take part in binding of either coenzyme or substrate (see page 979). Thus, the authors conclude on page 981 that Cys-255 is "unique". It is difficult to see in this reference, which touts the "uniqueness" of Cys-255 and does not suggest mutating Cys-5 at all, a motivation even to try making a double mutant at Cys-255 and Cys-5. And if one reading Tishkov et al. did decide to target a second cysteine in addition to Cys-255, why not mutate Cys-288, or any of the several other cysteines, instead of Cys-5? The Examiner has not explained just why one of ordinary skill in the art would choose to mutate exactly the residues specified in the claims, as opposed to some other combination of the seven cysteines of SEQ ID NO:2, or, for that matter, of the 394 non-cysteine residues of SEQ ID NO:2. **(Note in that regard that Galkin et al. found they could improve the thermostability of SEQ ID NO:2 by mutating Glu-61. This appears to teach away from any mutant forms of SEQ ID NO:2 that do not**

[1] In claims 41-46, there is a third mutation at Cys-146; the nonobviousness of such a mutation, and thus of claims 41-46, is discussed at length above.

include a mutation at Glu-61, with its known benefits. The Examiner has not addressed this teaching away in Galkin et al., which, Applicants note, is the primary reference cited.) And certainly none of the cited references gives the reader an expectation of success upon making the specifically claimed mutations, whether in the *Pseudomonas* enzyme or in SEQ ID NO:2 of the present invention.

To counter Applicant's point concerning lack of motivation to modify Cys-6 due to its likely being essential for activity, the Office action suggests that it "can be substituted with a serine residue because serine is a structural analogs of cysteine and the change gives information about the role of the sulfur atom in cysteine residues in formate dehydrogenase stability." Office Action at page 5. Applicants note in reply that such a "motivation"—i.e., to "give information"—does not rise to the level of what is needed to support an obviousness rejection. Any mutation in any protein could be said to "give information" of some sort about the role of the mutated residue in that protein. If the mere opportunity to collect experimental information about the effect of a given mutation were sufficient to render the resulting mutant protein obvious, then every mutant protein ever made would, *a priori*, be obvious. That is not the standard. There must be both a motivation to make *the particular claimed* mutation beyond scientific curiosity, as well as an expectation of success. Here, the prior art provides neither.

As a further note, Applicants point out that the above-quoted statement from the Office action about the motivation to substitute Cys-6 with serine ignores the fact that several claims specify substituting residues other than serine for Cys-6. See claims 36-38, 40, 45, and 46. Alanine and valine, which have hydrophobic side chains, are not considered "structural analogs" of cysteine, which is polar and hydrophilic. The Examiner has not explained the "motivation" for making these particular substitutions. Indeed, there is none.

Unexpected Results

In the prior response, Applicants explained that the presently claimed mutants possess a surprising characteristic: improved resistance to organic solvents. As an important commercial use of formate dehydrogenase requires the presence of an organic solvent such as 4-haloacetoacetate ester, the lower yield of the product due to decreased activity of the enzyme in

the commercial reaction was a significant problem. That problem is, quite surprisingly, addressed by the present invention. (See, e.g., Tables 2-4 of the specification.) The prior art did not know that decreased activity in the presence of organic solvent was the issue, nor that one could affect the activity in organic solvent by mutating certain residues of formate dehydrogenase.

The Examiner brushes this evidence aside, saying at page 4 of the Office action:

> The reaction medium of the reaction utilizing the enzyme is carried out in organic solvents. Also, the fact that appellant has recognized another advantage which would flow naturally from following the suggestion of the prior art cannot be the basis for patentability when the differences would otherwise be obvious.

It appears that the Examiner is saying that surprising results can never form the basis for overcoming a *prima facie* obviousness rejection, as such a rejection (assuming it is properly made) would mean that, by definition, "the differences would otherwise be obvious." This flies in the face of established law. See, for example, *In re Soni*, 54 F.3d 746, 750, 34 USPQ2d 1684, 1687 (Fed. Cir. 1995):

> One way for a patent applicant to rebut a prima facie case of obviousness is to make a showing of "unexpected results", i.e., to show that the claimed invention exhibits some superior property or advantage that a person of ordinary skill in the relevant art would have found surprising or unexpected. The basic principle behind this rule is straightforward—that which would have been surprising to a person of ordinary skill in a particular art would not have been obvious.

Thus, regardless of the strength of the *prima facie* case set forth in the Office action, it is certainly true, and unrebutted by the Examiner, that the prior art did not expect to find formate dehydrogenase mutants with improved activity in organic solvents compared to wildtype enzyme. According to the Federal Circuit, this fact alone is sufficient to confer patentability over the art. Furthermore, far from being just "another advantage," as the Examiner terms it, the advantage discovered by Applicants is the only advantage anyone has disclosed for the presently claimed mutant proteins, and is a commercially important one to boot.

In view of the above, Applicants respectfully request that the rejection be withdrawn and the claims allowed. Enclosed is a check for $288 for the required fee for excess claims. Please apply any other charges or credits to deposit account 06-1050.

Respectfully submitted,

Date: July 1, 2004

Janis K. Fraser, Ph.D., J.D.
Reg. No. 34,819

Fish & Richardson P.C.
225 Franklin Street
Boston, MA 02110-2804
Telephone: (617) 542-5070
Facsimile: (617) 542-8906
20877588.doc